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RESPIRATORY INFECTION AND SEPTICEMIA OF CATS DUE TO THE HEMOLYTIC STREPTOCOCCUS

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In February, 1921, an acute respiratory disease suddenly broke out among 25 cats kept in a room in this laboratory. The disease spread so rapidly and was so severe that all of these animals except 2 were dead within 15 days. This epizootic, having features in common with epidemics of respiratory disease in man, was an example of the spontaneous contagious infections occurring among laboratory animals which are becoming of increasing use to the experimental epidemiologist.[1]

The organism which caused the disease was a hemolytic streptococcus. Cats are said to be relatively insusceptible to infection with hemolytic streptococci, and a record of only one similar epizootic has been found in the literature. This occurred in a laboratory in Leyden, and was reported by de Jong,[2] in 1912. The "streptococcosis" of cats which he described appears to have been identical with the disease which occurred here. From his description, however, it is not possible to recognize the type of streptococcus which infected his animals.

The 25 cats among which the disease broke out here were kept as "stock" in a warm room. They were not in cages. As new cats were placed in this room at irregular intervals, it is not possible to say when the animal or animals introducing the infection were mixed with the others. When attention was directed to the condition, several cats were found to be sick, and in a few days all of the animals except 2 had become infected. The onset of the disease was characterized by sneezing, the appearance of a thin greenish discharge from the nose and by conjunctivitis. The infected animals soon became prostrated, lost weight rapidly, became exhausted and stuporous and died after 4 or 5 days. The cats had a leukocytosis of 50,000 to 100,000 cells per cubic millimeter. The percentage of polymorphonuclear neutrophils varied from 73 to 90.

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[1] Flexner, S.: Experimental Epidemiology, Jour. Exper. Med., 1922, 36, p. 9.

[2] Centralbl. f. Bakteriol., I, O., 1912, 66, p. 281.

At necropsy all of the cats presented the same general appearance. They were emaciated. A green mucopurulent exudate filled the cavities of the nose and extended over the nasopharynx down into the trachea as far as its bifurcation. The cranial sinuses were normal, and there were no gross or microscopic lesions attributable to this infection in any other organs. No evidence of pneumonia was found when the lungs were examined.

In smears of the exudate from the nose, pharynx and trachea, there were numerous gram-positive cocci in chains. This organism always predominated, and it was usually the only organism seen in the smears of the exudate from the trachea. Cultures of the exudate yielded many colonies of Streptococcus haemolyticus. Bacteriologic examination of the blood of the cats before and after their death revealed a more adequate basis for the severity of their symptoms. After the first day of the disease, all animals had a septicemia due to Streptococcus haemolyticus. Streptococci were sufficiently numerous in the blood to be recovered by plating a single drop of blood. Pure culture of the hemolytic streptococcus were obtained from the heart blood of these cats.

The disease, then, was characterized by an infection of the upper respiratory tract, followed shortly by a fatal septicemia. The absence of pneumonia was striking. Search for organisms of the hemorrhagic septicemia group was made. No evidence was obtained that such organisms were concerned in this infection. Streptococcus haemolyticus was predominant in the lesions and was not associated with any other bacterium in causing the septicemia.

Strains of this streptococcus were isolated from the trachea of 2 cats and from the blood of 7 cats. They were studied culturally and immunologically.[3]

STREPTOCOCCUS HAEMOLYTICUS FROM CATS

Morphology and Staining.—The streptococcus formed long chains of 8 or 16 or more cocci in meat infusion broth. The size of the cocci varied considerably, though not unusually. In some chains, the apposed sides of the cocci were flattened. All strains were gram-positive.

Cultural Studies.—The streptococcus grew well aerobically on blood agar, serum mediums and in meat infusion broth. On an agar plate containing 5% rabbit blood, the streptococcus formed a wide zone of clear hemolysis—the characteristic beta-type of Brown. When 1 c c of a 16 to 18-hour broth culture was added to 1 c c of 5% sheep cells, and incubated at 37 C., the cells were completely laked in a short time.

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[3] These strains were isolated by the late Dr. H. B. Cross. In the subsequent studies, the work of Miss Tuthill was of great assistance.

The fermentation reactions were determined by using mediums containing serum-water and 0.5% carbohydrate, with brom-cresol-purple as the indicator. These mediums were sterilized in the Arnold sterilizer on 3 days. The cultures were kept in the incubator at 37 C. for 2 weeks under daily observation. All strains fermented the same "sugars" —forming acid from dextrose, levulose, galactose, maltose, lactose, sucrose, salicin, raffinose, arabinose and dextrin. None fermented mannitol or inulin. These fermentation reactions are characteristic of Holman's Streptococcus pyogenes.

The final P_H produced by these streptococci was determined by growing them in 1% dextrose meat infusion broth. Each tube contained 10 c c of the medium, the initial reaction of which was P_H 7.8. These cultures were incubated at 37 C. for 24, 48 and 72 hours. Abundant growth occurred. After 48 hours the maximum acidity had been produced, and the reaction, determined colorimetrically with methyl red, was P_H 4.8. This reaction approaches that produced by the hemolytic streptococci from cattle.

Hydrolysis of Sodium Hippurate.—Recently, Ayers and Rupp[4] have shown that beta-hemolytic streptococci from bovine sources decompose sodium hippurate to benzoic acid and glycocoll while the beta-hemolytic streptococci of human origin are unable to effect this hydrolysis. The medium was prepared by adding 1% sodium hippurate to meat infusion broth containing a trace of ferric chloride. This was sterilized in the autoclave. All the strains of streptococci from cats and, as controls, a human strain and a characteristic bovine hemolytic streptococcus (No. M43 from Dr. F. S. Jones of the Department of Animal Pathology of the Rockefeller Institute) were inoculated into tubes of this medium. Tests for benzoic acid were made after the cultures had been growing for 24 hours. The reagents used for these tests were: (1) 12% ferric chloride solution in 2% hydrochloric acid and (2) 50% sulphuric acid. To 2 c c lots of the clear culture fluid, 0.5 c c of each reagent was added. The results were as follows:

1. Hemolytic streptococci from cats: No hydrolysis of sodium hippurate.

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[4] Jour. Infect. Dis., 1922, 30, p. 80.

2. Hemolytic streptococcus, human type: No hydrolysis of sodium hippurate.

3. Hemolytic streptococcus, bovine type, M_{43}: Hydrolysis, shown by persistent brown precipitate with ferric chloride and precipitation of crystals of benzoic acid with H_2SO_4.

Summary of Cultural Reactions.—All the strains of hemolytic streptococci isolated from the exudates and blood of these cats had identical cultural reactions. They caused the characteristic beta-type of clear hemolysis on blood agar, failed to ferment mannitol and inulin, produced a final P_H of 4.8, and were unable to hydrolize sodium hippurate. These reactions seem to exclude them from the group of bovine hemolytic streptococci but do not indicate what position they occupy in the scheme of classification.

IMMUNOLOGIC REACTIONS

Agglutination and the absorption of agglutinins were used in the attempt to discover the immunologic relationships of these streptococci from cats. As usual, it was difficult to make homogeneous suspensions of these organisms. Satisfactory suspensions were sometimes obtained with cultures in an infusion broth prepared from beef-heart. But the method of using cultures from ascites fluid agar, described by Herrold [5] was found to be much more satisfactory. Only homogeneous suspensions were used. As controls in the agglutination tests, a suspension of each organism in broth or salt solution was incubated with the other tubes in a water bath at 56 C. for 2 hours. Concentrated emulsions of streptococci grown on ascites fluid agar were used for the absorption tests. The following agglutinating serums were used:

1. Monovalent agglutinating serums for the human types of hemolytic streptococci, S 3, S 23 and S 84.[6] These were obtained from Dr. Dochez.

2. Monovalent agglutinating serum specific for a strain of hemolytic streptococcus from scarlet fever, prepared by Dr. Bliss.

3. Polyvalent antistreptococcus serum, from a horse, prepared by Dr. Huntoon.

4. Monovalent agglutinating serum for the bovine type (M_{43}) of hemolytic streptococcus, prepared by immunizing a rabbit to this strain. Titer 1:1,600.

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[5] Jour. Infect. Dis., 1922, 30, p. 80.

[6] Dochez, A. R.; Avery, O. T., and Lancefield, R. C.: Jour. Exper. Med., 1919, 30, p. 179.

5. Agglutinating serums specific for the hemolytic streptococci from cats, prepared by immunizing rabbits with many injections during about 6 weeks, as follows:

No. 153—antistreptococcus from blood of cat A; titer 1:5,120.
No. 154—antistreptococcus from trachea of cat A; titer 1:2,560.
No. 155—antistreptococcus from blood of cat B; titer 1:2,560.
No. 156—antistreptococcus from trachea of cat B; titer 1:1,280.
No. 157—antistreptococcus from blood of cat G; titer 1:2,560.

The results of the agglutination tests, with omission of the long protocols, are summarized in table 1.

These results show that the group of streptococci from these cats is composed of identical strains, and that they are immunologically different from human and bovine types of hemolytic streptococci. They form a cultural and serologic unit, exhibiting the homogeneity of an "epidemic strain," which is entirely concordant with their unvarying effect on the animals from which they were isolated.

TABLE 1

AGGLUTINATION REACTIONS OF HEMOLYTIC STREPTOCOCCI FROM CATS

Serum	Result
Antistreptococcus serum:	
Human Type S 3	No agglutination of streptococci from cats.
Human Type S 23	No agglutination of streptococci from cats.
Human Type S 84	No agglutination of streptococci from cats.
Human scarlatinal Type	No agglutination of streptococci from cats.
Polyvalent serum (Huntoon)	No agglutination of streptococci from cats.
Bovine type (M_{43})	No agglutination of streptococci from cats.
No. 153, cat A, blood	Agglutinated all strains from cats.
No. 154, cat A, trachea	Agglutinated all strains from cats.
No. 155, cat B, blood	Agglutinated all strains from cats.
No. 156, cat B, trachea	Agglutinated all strains from cats.
No. 157, cat G, blood	Agglutinated all strains from cats.

The absorption of agglutinins confirmed the results of the agglutination reactions. To absorb the agglutinins, a thick emulsion of streptococci was made by washing off the cultures from slants and plates of ascites fluid agar with salt solution and by centrifuging this. The antiserums were diluted 1:20, and 2 volumes of serum were mixed with 1 volume of sedimented streptococci. This mixture was incubated in a water bath at 56 C. for 2 hours. The serum was then freed from the organisms by centrifugation, and used for agglutination tests. The results of these are summarized in table 2.

Pathogenicity.—The streptococci soon lost the ability to produce the disease when placed within the noses of cats. Their pathogenicity for cats, however, was retained through a number of transfers in broth. Three·cc of an 18-hour broth culture, injected intravenously, produced septicemia which killed the animals in 72 hours. The opportunity has not presented itself for an extended "epidemiologic" study with this streptococcus.

Incidence of Streptococcus in Cats.—To determine the frequency of the occurrence of streptococci in the throats of cats, 50 normal animals were studied. Some of the animals were taken from the stockroom, others used as soon as they were brought into the laboratory from the streets. The throats were swabbed and the material thus obtained was plated on 5% rabbit's blood agar. A beta-hemolytic streptococcus was found in only 1 of these cultures, showing again that streptococci occur only rarely in the nasopharynx of cats (0.5%).

TABLE 2

RESULT OF AGGLUTINATION TESTS

Antistreptococcus Serum	Absorbed with	Agglutination Test after Absorption	Result
153 antistreptococcus Cat A, blood	Streptococcus from cat A blood	All strains of streptococci from cats	Removed agglutinins for all strains of streptococci from cats
153 antistreptococcus Cat A, blood	Human Type streptococcus	All strains of streptococci from cats	Slight, insignificant reduction of agglutins
153 antistreptococcus Cat A, blood	Bovine type streptococcus M_{43}	All strains of streptococci from cats	Slight, insignificant reduction of agglutins

SUMMARY

An unusual epizootic in cats due to a variety of hemolytic streptococcus is described. The disease began as an infection of the upper respiratory tract and ended with septicemia. Pneumonia did not occur. The infection spread rapidly among associated animals and had a high mortality.

The organism causing the disease, Streptococcus haemolyticus, resembled the human types of hemolytic streptococci more closely than the bovine, but different immunologically from the strains of both of these varieties, with which they were compared. All the strains of this streptococcus were identical.